PhosphorImager.

In the specific examples shown in Figures 2 and 4, experimental conditions were as follows:

$tRNA^{phe}$ (10 pmol, Sigma) was dissolved in HEPES buffer (50mM, pH7.4; 20mM $MgCl_2$, 3.3mM DTT, 1μM ATP, 10 μg/ml BSA, 10% DMSO) with cytosine-3',5'-diphosphate (3000Ci/mmol, Amersham), and T4 RNA ligase (9 units, Pharmacia), after 30min incubation at 37°, the reaction was separated in a spun column and the labelled RNA dissolved in 3.5M TMA (10ml). Hybridisation was carried out by applying ca. 1ml of the solution to the surface of the array and overlaying a second glass plate of the same dimensions. The "sandwich" was placed in a sealed box at 4°C for 18-24h. The plates were separated, the array rinsed in the hybridisation solvent at 4°C, and analysed as described above.

For the cooperative experiments shown in Figure 4, cold oligonucleotides corresponding to the D-loop GCTCTCCCAACT, (SEQ ID No. 1) the variable loop GACCTCCAGATT, (SEQ ID No. 2) or the TpsiC loop AACACAGGACCT, (SEQ ID No. 3) were incubated with the tRNA in the hybridisation conditions for at least 18h before being applied to the plate.

FIGURES

Figure 1.

An illustrative array comprising all tetranucleotide sequences. This is one sixteenth the size of the smallest usable array comprising all hexanucleotides, which would be too complex to display in this format. The letters along the top and down the left hand side show the order in which base precursors were applied in columns and rows during the synthesis of the oligonucleotides. The letters in each cell show the sequence of the oligonucleotide synthesised in that cell. Larger arrays are made by simply ovelaying

glass through the 3' ends.

b) natural deoxyribonucleotides tethered to the glass through the 5' ends.

c) deoxyribophosphothioates (Note that this array was exposed in the opposite orientation to the other three).

d) ribonucleotides.

The arrays were all hybridised under identical conditions (3.5 M tetramethylammonium chloride, 4°C) with the sequence CCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTTCCTAT, (SEQ ID No. 4) part of exon 10 of the CFTR gene covered by the array.

The deoxyribonucleotides give essentially the same result in both chemical orientations, but the analogues, the deoxyribophosphothioates and ribonucleotides, give quite different results. Although a difference may be expected, this experiments shows the difficulty in extrapolating data from one analogue to others, and further demonstrates the power of the array technique in identifying candidates for antisense reagents, including analogues.

Figure 7.

The Rev response element (RRE) of HIV is considered to be a good potential target for therapeutic intervention by antisense oligonucleotides because of its central place in the regulation of gene expression from the viral genome. However, molecular modelling in the computer, as shown (Fig. 7a), suggests that this region of HIV RNA is likely to have a complex folded structure, and this has been confirmed by analysing the susceptibility of the RNA to nucleases. This structure makes it difficult to select regions for antisense targetting. We have analysed the hybridisation behaviour in a two stage process. First, the labelled RNA was hybridised to "universal" arrays,